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**Geographic variations in phenotypic traits of the exotic herb *Solidago altissima*
and abundance of recent established exotic herbivorous insects**

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14 **Abstract**

15 Many invasive plants increase aggressiveness after introduction. Since
16 evolutionary forces such as herbivore pressure may change over different time scales,
17 understanding the changes in biotic interactions in invasive plants through time can
18 clarify the mechanism of their evolution in aggressiveness. In this study we examined
19 the geographic variation in phenotypic traits of *Solidago altissima* and the abundance of
20 two exotic herbivorous insect species (the aphid, *Uroleucon nigrotuberculatum* and the
21 lacebug, *Corythucha marmorata*), which are recently expanding their habitat on *S.*
22 *altissima* populations over Japan. The two exotic insects were present at high density on
23 *S. altissima* throughout their range. No differences in growth traits (plant height and
24 number of leaves) were found among populations, and all plants examined appear to be
25 exclusively hexaploid. Future studies on population genetics and common garden
26 experiments are necessary to evaluate the potential evolutionary dynamics of the *S.*
27 *altissima* after introduction.

28

29 **Keywords**

30 *Corythucha marmorata*, exotic insects, hexaploid, species invasion, *Solidago altissima*,

31 *Uroleucon nigrotuberculatum*

32 **Introduction**

33 Rapid evolution has been found in many successful invasive plants, including
34 changes in traits such as biomass, reproductive output, competitive and dispersal
35 abilities (e.g. Blossey & Notzold 1995, Maron et al. 2004, Brown & Eckert 2005). The
36 enemy release hypothesis (ERH), one of the influential hypotheses considered as
37 fundamental in explaining plant invasion success, is important by transporting the plant
38 away from its natural enemies allowing the plant to grow vigorously. Many studies
39 examining traits of invasive plants have focused on a snapshot in time, however,
40 evolutionary forces such as herbivore pressure may change over different time scales
41 (Hawkes 2007). Understanding the pattern of changes in potential drivers of invasive
42 plants through time can clarify how invaders continue to be successful.

43 A perennial herb, *Solidago altissima*, was introduced to Japan in the early
44 19th century from North America, and has expanded its distribution rapidly over Japan
45 after the 1960s (Shimizu 2003). *Solidago altissima* in North America consists of diploid,

46 tetraploid, and hexaploid ($2n=18, 36, 54$) (Halverson et al. 2008a). While the taxonomic
47 treatment of the species has been complex, recent treatments (Semple and Cook 2006)
48 have recognized two subspecific taxa. These subspecific taxa is associated with
49 cytotypic variation, with subsp. *gilvocanescens* reported as diploid and tetraploid across
50 its range and subsp. *altissima* primarily hexaploid (a few tetraploids have been reported
51 at the western edge of the distribution and across the southeastern US; Semple and
52 Cook, 2006). However, the ploidy level in Japan is unknown. Although it is considered
53 as one of the most invasive, of introduced plants in Japan, ecological traits and natural
54 enemies throughout its distributional range have been poorly explored. Recently, two
55 exotic insects have been introduced to Japan from North America: the aphid *Uroleucon*
56 *nigrotuberculatum* in 1990's and the lacebug *Corythucha marmorata* in 2000. The aphid
57 has a large impact on the native insect community via changing *S. altissima* traits (Ando
58 et al. 2011). On the other hand, the lacebug has been rapidly expanding its habitat, and
59 has become a serious pest of crops such as chrysanthemum and sweet potato in Japan.
60 The aim of this study was to elucidate the geographic variation in the abundance of the
61 two exotic herbivorous insect species among *S. altissima* populations over Japan.

Because the abundance of the herbivorous insects is dependent on plant traits such as plant size (Lawton 1983) and ploidy levels (Halverson 2008b), we also examined plant traits related to the abundance of the two insects. This is fundamentally important for understanding the changes of the herbivore pressure on *S. altissima* after expansion in the invasive range.

Methods

In June in 2011 and 2012, we surveyed the abundance of the two exotic insects at 15 sites (1-5 *S. altissima* populations per site) in Japan (Table 1). Populations at each site occur within radius 10 km, and the distance of two adjacent populations was 1 km. We surveyed 5-10 individual plants (three ramets per individual) distinguished by clumps in each population. For each ramet, number of the two insects was counted.

We recorded plant height and number of leaves for all ramets which the insect survey was conducted. Then, we collected rhizomes from five individuals of one population at each site for determination of the ploidy level. Ploidy levels were determined by flow cytometry and chromosome numbers (chromosome counts in root

tip squashes of the cultivated plants from rhizomes). The root tips were treated with a 0.05% hydroxynole solution at 16-18°C for 5 h before they were fixed with an ethanol : glacial acetic acid solution (3 : 1) at 4°C for 24 h. They were macerated by 1N HCL at 60°C for 1 min before being stained with aceto-carmine solution for 24 h and were mounted on a microscope slide. In the flow cytometry analysis, for each sample intact nuclei were extracted from approximately 0.5 cm² of leaf tissue in a Petri dish. The sample was chopped for 30 sec using a sharp steel razor blade in 400 µl of extraction buffer (Partec, Görlitz, Germany) and filtered using a 30-µm CellTrics disposable filter (Partec). For each sample, the filtrate was mixed with 1.6 ml of staining solution (Partec) and the mix was incubated for 60 s. We analyzed these samples, using CyStain UV precise P (Partec). We converted fluorescence to chromosome number, using standard samples (hexaploid samples determined by root tip squashes). Only samples producing a histogram peak with a low coefficient of variation (< 5%) were retained. The standard sample was checked after every five samples.

Results & Discussion

Both the lacebugs and the aphids were observed in high densities while native

94 generalist herbivores including grasshoppers and geometric moth larvae were rare.
95 Although there was a considerable variation in the aphid abundance among populations,
96 aphids were found in all sites (Table 1). On the other hand, the lacebugs were absent in
97 sites of Hokkaido and Sado, indicating that they have not invaded those sites yet (Table
98 1). The effect of the latitude was marginally significant on the abundance of the
99 lacebugs and it was greater in the lower latitudinal populations [generalized linear
100 mixed model (GLMM): random effects = individual nested with population, offset =
101 leaf number (as plant size), $z = -1.82$, $df = 343$, $P = 0.07$], while greater aphid
102 abundance was apparent in higher latitudinal populations [GLMM: random effects =
103 individual nested with population, offset = plant height (as plant size), $z = 2.91$, $df =$
104 343 , $P = 0.004$].

105 No latitudinal clinal patterns were found in both plant height [GLMM:
106 random effects = individual nested with population, $z = -1.38$, $df = 343$, $P = 0.17$] and
107 leaf number [GLMM: random effects = individual nested with population, $z = -0.032$, df
108 $= 343$, $P = 0.975$]. Flow cytometry analyses mostly yielded high-resolution histograms,
109 with average sample CV of 3.25% (range 1.99–4.86%). Flow cytometry data for 75

110 individuals of *S. altissima* from 15 sites showed that all individuals had the same value.
111 This indicates that all individuals of *S. altissima* examined in the present study had the
112 same ploidy level and they were hexaploid ($2n = 54$) (Table 1). Therefore, they were all
113 subsp. *altissima*.

114 Our field survey suggests that the two exotic insects were dominant
115 herbivores on *S. altissima*. In particular, the lacebugs may have a selective impact to the
116 traits of *S. altissima* because they continue causing severe damage to the plant by
117 sucking the leaf tissue until the end of autumn. Moreover, because *C. marmorata* is
118 expanding its range concentrically, the dates of population establishment differ
119 geographically (including absent sites). This provides an excellent opportunity to test
120 the potential of the selective impacts of this herbivore on traits of *S. altissima*. Although
121 no differences in plant height and leaf number among populations indicate no difference
122 in plant growth traits throughout the range, other traits such as reproduction and
123 resistance may differ among populations with different abundance of the exotic insects.
124 There are two possible explanations for the result that only hexaploid plants were found.
125 One is that only hexaploid plants had been introduced. Another is that other ploidies had

been also introduced, but they failed to establish or expand its ranges. Studies of the ploidy level of *S. gigantea* in the invasive and native range revealed that tetraploids were more invasive than diploids and it was the only cytotype found in the invasive range (Schlapfer et al. 2008; 2010). Future research on population genetics and common garden experiments is necessary to evaluate the potential evolutionary dynamics of *S. altissima* after introduction.

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171

Table 1. Geographical information of *S. altissima* populations and the means (± 1 SE) of two traits (height and no. leaves), ploidy level and mean number (± 1 SE) of two exotic insects.

Site	No.	Latitude	Longitude	Height	No. leaves	Ploidy		
	populations					level	No. lacebugs	No. aphids
Hokkaido	5	42.83	141.30	99.45 \pm 3.34	32.48 \pm 1.02	Hexaploid	0	34.76 \pm 9.00
Tochigi	2	36.67	139.95	80.10 \pm 8.38	38.00 \pm 3.26	Hexaploid	13.10 \pm 3.79	14.80 \pm 8.09
Sado	2	37.80	138.24	85.32 \pm 3.25	35.34 \pm 1.59	Hexaploid	0	2.34 \pm 1.69
Nigata	2	37.88	139.04	92.86 \pm 4.54	28.33 \pm 1.15	Hexaploid	9.00 \pm 2.68	1.79 \pm 1.79
Tokyo	2	35.65	139.65	82.98 \pm 2.25	31.10 \pm 1.12	Hexaploid	8.67 \pm 2.56	33.20 \pm 15.23
Sizuoka	1	35.13	138.64	92.33 \pm 5.56	33.38 \pm 1.66	Hexaploid	3.85 \pm 0.96	54.05 \pm 19.52
Kyoto	3	34.84	135.53	95.44 \pm 4.22	28.56 \pm 1.70	Hexaploid	6.61 \pm 1.39	4.55 \pm 2.48
Shiga	2	34.80	135.66	96.87 \pm 3.28	37.73 \pm 1.49	Hexaploid	8.86 \pm 2.08	32.10 \pm 9.61
Osaka	3	34.90	135.45	88.29 \pm 3.54	34.35 \pm 1.31	Hexaploid	4.3 \pm 0.77	17.22 \pm 5.16
Hyogo	5	35.20	135.23	84.22 \pm 2.24	34.17 \pm 1.00	Hexaploid	7.29 \pm 0.84	20.66 \pm 4.76
Kochi	1	33.56	133.56	119.24 \pm 4.07	21.84 \pm 1.29	Hexaploid	1.32 \pm 0.39	1.05 \pm 3.04
Fukuoka	4	33.62	130.37	96.98 \pm 2.43	37.33 \pm 1.07	Hexaploid	4.78 \pm 0.76	12.64 \pm 2.04
Saga	3	33.32	130.27	83.89 \pm 3.34	42.81 \pm 2.25	Hexaploid	8.25 \pm 2.18	41.17 \pm 2.15
Kumamoto	4	32.92	130.8	100.44 \pm 2.26	37.82 \pm 1.18	Hexaploid	15.67 \pm 2.25	11.50 \pm 4.39
Kagoshima	3	31.65	130.47	110.54 \pm 2.89	33.09 \pm 1.16	Hexaploid	14.88 \pm 2.20	3.86 \pm 1.79